



KONGERIKET NORGE  
The Kingdom of Norway

Bekreftelse på patentsøknad nr  
*Certification of patent application no*




20034952

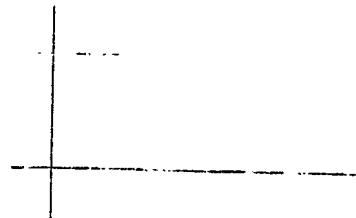
30.11.2003

▷ Det bekreftes herved at vedheftede dokument er nøyaktig utskrift/kopi av ovennevnte søknad, som opprinnelig inngitt 2003.11.06

▷ *It is hereby certified that the annexed document is a true copy of the above-mentioned application, as originally filed on 2003.11.06*

2005.01.19

  
Ellen B. Olsen  
Saksbehandler



Medl: KH, Int. U: 007K<sup>47</sup> 23136019

2003 -11- 06

www.patentstyret.no

Alm. tilgj. 09. MAI 2005

erdig utfylt skjema sendes til adressen nedenfor. Vennligst ikke heft sammen sidene.  
Ber om at blankettene utfylles *maskinelt* eller ved bruk av *blokkbokstaver*. Skjema for  
tfilling på datamaskin kan lastes ned fra [www.patentstyret.no](http://www.patentstyret.no).

1a-f

**Søker:** Den som søker om patentbrev også innehar av en eventuell rettighet. Må fylles ut.

Foretakets navn (fornavn hvis søker er person):

Etternavn (hvis søker er person):

Amersham Health AS

Oppgi gjerne kundenummer:

☒ Kryss av hvis søker tidligere har vært kunde hos Patentstyret.

Adresse:

Nycoveien 1-2

Postboks 4220 Nydalen

Postnummer:

0401

Poststed:

Oslo

Land:

Norge

☐ Kryss av hvis flere søkere er angitt i medfølgende skjema eller på eget ark.

☐ Kryss av hvis søker(ne) utfører mindre enn 20 årsverk (se veiledning).

☐ Kryss av hvis det er vedlagt erklæring om at patentsøker(ne) innehar retten til oppfinnelsen.

**Kontaktinfo:** Hvertisk Patentstyret henvende seg til? Oppgi telefonnummer og eventuell referanse.

Fornavn til kontaktperson for fullmektig eller søker:

Etternavn:

Tove Aas

Helge

Telefon:

2 3 1 8 5 5 7 5

Referanse (maks. 30 tegn):

PN0380-NO

**Evt. adresse til kontaktperson:**

Amersham Health AS

Nycoveien 1-2, Postboks 4220 Nydalen

Postnummer:

0401

Poststed:

Oslo

Land:

Norge

**Fullmektig:** Hvis du ikke har opplyst en fullmektig, kan du gå til neste punkt.

Foretakets navn (fornavn hvis fullmektig er person):

Etternavn (hvis fullmektig er person):

Amersham Health AS

☒ Kryss av hvis fullmektig tidligere har vært kunde hos Patentstyret.

Oppgi gjerne kundenummer:

Adresse:

Nycoveien 1-2

Postboks 4220 Nydalen

Postnummer:

0401

Poststed:

Oslo

Land:

Norge

**Oppfinner:** Oppfinneren skal alltid oppgis selv om oppfinner og søker er samme person.

Oppfinnerens fornavn:

Etternavn:

Alan

Cuthbertson

☐ Kryss av hvis oppfinner tidligere har vært kunde hos Patentstyret.

Oppgi gjerne kundenummer:

Adresse:

Amersham Health AS

Nycoveien 1-2, Postboks 4220 Nydalen

Postnummer:

0401

Poststed:

Oslo

Land:

Norge

☒ Kryss av hvis flere oppfinnere er angitt i medfølgende skjema eller på eget ark.

ADRESSE

Postboks 8160 Dep.  
København 10  
0033 Oslo

TELEFON

22 38 73 00

TELEFAKS

22 38 73 01

BANKGIRO

8276.01.00192  
ORGANISASJONSNR.  
871526157 MVA



**PATENTSTYRET**  
Styret for det industrielle rettsvern

SØKNAD S. 1 AV 2

FLERE SØKERE

FLERE OPPFINNERE

PRIORITETER

VEILEDNING



**Tittel:** Gjør en kort benevnelse eller tittel for oppfinnelsen (ikke over 256 tegn, inkludert mellomrom).

Tittel:  
Farmasøytiske forbindelser

**PCT:** Fylls bare ut hvis denne søknaden er en videreføring av en tidligere innlevert internasjonal søknad (PCT).

Inngivelsesdato (åååå.mm.dd):

Søknadsnummer:

PCT-søknadens dato og nummer:

PCT /

**Prioritetskrav:** Hvis du ikke har søkt om denne oppfinnelsen tidligere (i et annet land eller i Norge) kan du gå videre til neste punkt.

**Prioritet kreves på grunnlag av tidligere innlevert søknad i Norge eller utlandet:**

Inngivelsesdato (åååå.mm.dd):

Landkode:

Søknadsnummer:

Opplysninger om tidligere søknad. Ved flere krav skal tidligste prioritet angis her:

☐ Flere prioritetskrav er angitt i medfølgende skjema, eller på eget ark.

**Mikroorganisme:** Fylls bare ut hvis oppfinnelsen omhandler en mikroorganisme.

**Søknaden omfatter en kultur av mikroorganisme. Deponeringssted og nummer må oppgis:**

Deponeringssted og nummer (benytt gjerne eget ark):

☐ Prøve av kulturen skal bare utleveres til en særlig sakkyndig.

**Avdelt/utskilt:** Hvis du ikke har søkt om patent i Norge tidligere, kan du gå videre til neste punkt.

**Søknaden er avdelt eller utskilt fra tidligere levert søknad i Norge:**

☐ Avdelt søknad

Informasjon om opprinnelig

Dato (åååå.mm.dd):

Søknadsnummer:

☐ Utskilt søknad

søknad/innsendt tilleggsmateriale

**Annnet:**

☒ Søknaden er også levert per telefaks.

Oppgi dato (åååå.mm.dd):

2-0-0-3-1-1-0-6

☐ Jeg har badt om forundersøkelse.

Oppgi nr (årstall - nummer - bokstav):

**Vedlegg:** Angi hvilken dokumentasjon av oppfinnelsen du legger ved, samt andre vedlegg.

☒ Eventuelle tegninger i to eksemplarer

Oppgi antall tegninger: 1

☒ Beskrivelse av oppfinnelsen i to eksemplarer

☒ Patentkrav i to eksemplarer

☒ Fullmaktsdokument(er)

☐ Sammendrag på norsk i to eksemplarer

☐ Overdragelsesdokument(er)

☐ Dokumentasjon av eventuelle prioritetskrav (prioritetsbavis)

☐ Erklæring om retten til oppfinnelsen

☐ Oversettelse av internasjonal søknad i to eksemplarer (kun hvis PCT-felt over er fylt ut)

**Dato/underskrift:** Sjekk at du har fylt ut punktene under. Søker: Oppfinner: og Vedlegg: Signer søknaden.

Signatur:

*Sove for følge*

Sted og dato (blokkbokstaver):  
Oslo, 6. november 2003

Navn i blokkbokstaver:

Tove Aas Helge

NB! Søknadsavgiften vil bli fakturert for alle søknader (dvs. at søknadsavgiften ikke skal følge søknaden).  
Betalingsfrist er ca. 1 måned, se faktura.



**PATENTSTYRET**  
Styret for det industrielle rettvern

SØKNAD - s. 2 av 2

Vedleggsskjema:

www.patentstyret.no



Dette skjemaet benyttes som vedlegg til patentsøknaden for å oppgi flere oppfinnere. **NB! Gi hver oppfinner et nummer. Personen oppgitt på søknadsskjemaet vil alltid bli registrert som nr. 01. Første angivelse på dette skjema vil være oppfinner 02. Skjema for utfylling på datamaskin kan lastes ned fra [www.patentstyret.no](http://www.patentstyret.no).**

Referanse:

Gjenta referansen fra «kontaktinnr.» eller tittel søkerens navn, som angitt på søknadsskjemaets første side. Må fylles ut.

Referanse:

PN0380-NO

Oppfinner nr. 0 2

Fornavn og mellomnavn:

Bård

Etternavn:

Indrevoll

Oppgi gjerne kundennummer:

☐ Oppfinner har tidligere vært kunde hos Patentstyret.

Adresse:

Amersham Health AS

Nycoveien 1-2, Postboks 4220 Nydalen

Postnummer:

0401

Poststed:

Oslo

Land:

Norge

Oppfinner nr.

Fornavn og mellomnavn:

Etternavn:

☐ Oppfinner har tidligere vært kunde hos Patentstyret.

Adresse:

Oppgi gjerne kundennummer:

Postnummer:

Poststed:

Land:

Oppfinner nr.

Fornavn og mellomnavn:

Etternavn:

☐ Oppfinner har tidligere vært kunde hos Patentstyret.

Adresse:

Oppgi gjerne kundennummer:

Postnummer:

Poststed:

Land:

Oppfinner nr.

Fornavn og mellomnavn:

Etternavn:

☐ Oppfinner har tidligere vært kunde hos Patentstyret.

Adresse:

Oppgi gjerne kundennummer:

Postnummer:

Poststed:

Land:

NB! Ved behov for mer plass benyttes flere skjema eller eget ark.



**PATENTSTYRET®**  
Styret for det industrielle rettsvern

FLERE OPPFINNERE

PATENTSTYRET

PHARMACEUTICAL COMPOUNDS

03-11-06\*20034952

Field of invention

The present invention provides novel pharmaceuticals useful in the treatment of heart failure, cardiac arrhythmia and other diseases where fibrosis is prominent and diagnosis of diseases and conditions where a fibrotic process is prominent. The invention further provides novel pharmaceutical compositions and precursors for the preparation of diagnostic agents. Further the invention provides novel pharmaceuticals useful in monitoring therapeutic treatment and methods for monitoring of treatment. Still further objectives of the invention are evident from the claims.

The novel pharmaceuticals comprise a targeting moiety that binds to a receptor that may or may not be up-regulated and/or over-expressed in the diseased area. The targeting moiety comprises a group which for imaging agents carry a diagnostically imageable moiety, an optional linker group and a peptide moiety.

The novel pharmaceutical compounds have high affinity for the Angiotensin Receptors, in particularly for the Angiotensin II (Ang II) type 1 (AT<sub>1</sub>) receptor.

Background of Invention

Angiotensin II (Ang II) - the octapeptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) - is a pleiotropic vasoactive peptide that binds to two distinct receptors: the Ang II type 1 (AT<sub>1</sub>) and type 2 (AT<sub>2</sub>) receptors. Activation of the renin-angiotensin aldosterone system (RAAS) results in vascular hypertrophy, vasoconstriction, salt and water retention, and hypertension. These effects are mediated predominantly by AT<sub>1</sub> receptors. Paradoxically, other Ang II-mediated effects, including cell death, vasodilation, and natriuresis, are mediated by AT<sub>2</sub> receptor activation. The understanding of Ang II signalling mechanisms remains incomplete. AT<sub>1</sub> receptor activation triggers a variety of intracellular systems, including tyrosine kinase-induced protein phosphorylation, production of arachidonic acid metabolites, alteration of reactive oxidant species activities, and fluxes in intracellular Ca<sup>2+</sup> concentrations. AT<sub>2</sub> receptor activation leads to stimulation of bradykinin, nitric oxide production, and prostaglandin metabolism, which are, in large part, opposite to the effects of the AT<sub>1</sub> receptor.

(See: Berry C, Touyz R, Dominiczak AF, Webb RC, Johns DG.: Am J Physiol Heart Circ Physiol. 2001 Dec;281(6):H2337-65.

Angiotensin receptors: signalling, vascular pathophysiology, and interactions with ceramide).

Ang II is the active component of the renin-angiotensin-aldosterone system (RAAS). It plays an important physiological role in the regulation of blood pressure, plasma volume, sympathetic nervous activity, and thirst responses. Ang II also has a pathophysiological role in cardiac hypertrophy, myocardial infarction, hypertension, chronic obstructive pulmonary disease, liver fibrosis and atherosclerosis. It is produced systemically via the classical RAAS and locally via tissue RAAS. In the classical RAAS, circulating renal-derived renin cleaves hepatic-derived angiotensinogen to form the decapeptide angiotensin I (Ang I), which is converted by angiotensin-converting enzyme (ACE) in the lungs to the active Ang II. Ang I can also be processed into the heptapeptide Ang-(1-7) by tissue endopeptidases.

The RAAS system is illustrated schematically in Figure 1 hereto which is based on Figure 1 in the article by Foote et al. in Ann. Pharmacother. 27: 1495-1503 (1993).

In addition to the RAAS playing an important role in the normal cardiovascular homeostasis, over activity of the RAAS has been implicated in the development of various cardiovascular diseases, such as hypertension, congestive heart failure, coronary ischemia and renal insufficiency. After myocardial infarction (MI), RAAS becomes activated. Specifically the AT<sub>1</sub> receptor seems to play a prominent role in post-MI remodelling, since AT<sub>1</sub> receptor expression is increased after MI and in left ventricular dysfunction. Therefore drugs that interfere with RAAS, such as ACE inhibitors and AT<sub>1</sub> receptor antagonists, have been shown to be of great therapeutic benefit in the treatment of such cardiovascular disorders.

For heart, kidneys, lungs and liver alike, fibrosis represents a common pathway to their failure. Understanding pathophysiologic mechanisms involved in organ fibrosis are therefore of considerable interest, particularly given the potential for protective pharmacological strategies. Tissue repair involves inflammatory cells, including members of the monocyte/macrophage lineage, integral to initiating the repair process; and myofibroblasts, phenotypically transformed interstitial fibroblasts, responsible for collagen turnover and fibrous tissue formation. Each of these cellular events in the microenvironment of repair are associated with molecular events that

lead to the de novo generation of angiotensin II (Ang II). In an autocrine/paracrine manner, this peptide regulates expression of TGF-beta 1 via angiotensin (AT<sub>1</sub>) receptor-ligand binding. It is this cytokine that contributes to phenotypic conversion of fibroblasts to myofibroblasts (myoFb) and regulates myofibroblast turnover of collagen. Angiotensin-converting enzyme (ACE) inhibition or AT<sub>1</sub> receptor antagonism each prevent many of these molecular and cellular responses that eventuate in fibrosis and therefore have been found to be protective interventions. (See: Weber KT. Fibrosis, a common pathway to organ failure: angiotensin II and tissue repair. *Semin Nephrol.* 1997 Sep;17(5):467-91 and references therein).

Ang II may regulate tissue fibrosis via the activation of mesenchymal cells. For example, Ang II stimulates the proliferation of cardiac fibroblasts *in vitro* via activation of AT<sub>1</sub>. The presence of AT<sub>1</sub> receptors has also been demonstrated on cardiac fibroblasts *in vitro*. Most of the profibrotic effects of Ang II appear to be mediated via this receptor; however, increased AT<sub>2</sub> expression on cardiac fibroblasts has been detected in hypertrophied human heart, and the balance between the expression of these two subtypes may be critical in determining the response to Ang II.

(See: *Am. J. Respir. Crit. Care Med.*, Volume 161, Number 6, June 2000, 1999-2004 Angiotensin II Is Mitogenic for Human Lung Fibroblasts via Activation of the Type 1 Receptor RICHARD P. MARSHALL, ROBIN J. MCANULTY, and GEOFFREY J. LAURENT and references therein).

The Ang II receptors can be distinguished according to inhibition by specific antagonists. AT<sub>1</sub> receptors are selectively antagonized by biphenylimidazoles, such as Losartan, whereas tetrahydroimidazopyridines specifically inhibit AT<sub>2</sub> receptors. The AT<sub>2</sub> receptor may also be selectively activated by CGP-42112A. This is a hexapeptide analog of Ang II, which may also inhibit the AT<sub>2</sub> receptor, depending on concentration). Two other angiotensin receptors have been described: AT<sub>3</sub> and AT<sub>4</sub> subtypes.

In rodents, the AT<sub>1</sub> receptor has two functionally distinct subtypes, AT<sub>1A</sub> and AT<sub>1B</sub>, with >95% amino acid sequence homology.

The second major angiotensin receptor isoform is the AT<sub>2</sub> receptor. It has low amino acid sequence homology (~34%) with AT<sub>1A</sub> or AT<sub>1B</sub> receptors. Although the exact signaling pathways and the functional roles of AT<sub>2</sub> receptors are unclear, these receptors may antagonize, under physiological conditions, AT<sub>1</sub>-mediated actions

inhibiting cell growth and by inducing apoptosis and vasodilation. The exact role of  $AT_2$  receptors in cardiovascular disease remains to be defined.

Other receptors for Ang II besides  $AT_1$  and  $AT_2$  are known and are generally referred to as  $AT_{\text{atypical}}$  (see Kang et al., Am. Heart J. 127: 1388-1401 (1994)).

The suppression of Ang II's effects has been used therapeutically, for example in the management of hypertension and heart failure. This has been achieved in a number of ways: by the use of renin inhibitors which block the conversion of angiotensinogen to angiotensin I (the precursor to Ang II); by the use of angiotensin converting enzyme (ACE) inhibitors that block the conversion of angiotensin I to Ang II (and also block bioconversion of bradykinin and prostaglandins); by the use of anti-Ang II - antibodies; and by the use of Ang II -receptor antagonists.

Beta blockers are most commonly used in treatment of arrhythmias. Anti-arrhythmic drugs have had limited overall success and calcium channel blockers can sometimes induce arrhythmias. No single agent shows superiority, with the possible exception of amiodarone. Short-term anti-arrhythmic benefit has been found to be offset by, depending on the specific drug, neutral or negative effects on mortality (Sanguinetti MC and Bennett, PB: Anti-arrhythmic drug target choices and screening. Circulation 2003, 93(6): 491-9257-263). Clearly better anti-arrhythmic drugs are needed.

A publication in Lancet (Lindholm, LH et al. Effect of Losartan on sudden cardiac death in people with diabetes: data from the LIFE study. The Lancet, 2003, 362: 619-620) revealed that  $AT_1$  receptor antagonists in addition of being generally favourable to patients with CHF, also reduce the incidence of sudden cardiac death. There exist a few studies showing that  $AT_1$  antagonists have an anti-arrhythmia effect on arrhythmias induced by myocardial infarct or in reperfusion after ligation of LAD (Harada K et al. Angiotensin II Type 1a Receptor is involved in the occurrence of reperfusion arrhythmias. Circulation. 1998,97:315-317. Ozer MK et al. Effects of Captopril and Losartan on myocardial ischemia-reperfusion induced arrhythmias and necrosis in rats. Pharmacological research, 2002, 45 (4), 257-263 Lynch JJ et al. EXP3174, the  $AT_1$  antagonist human metabolite of Losartan, but not Losartan nor the Angiotensin-converting enzyme inhibitor captopril, prevents the development of lethal ischemic arrhythmias in a canine model of recent myocardial infarction. JACC, 1999, 34 876-884).

### Description of Related Art

WO 98/18496 (Nycomed Imaging AS) discloses contrast agents comprising a vector-linker-reporter construct where the vector comprises angiotensin or a peptidic angiotensin derivative.

US patent 4411881 (New England Nuclear Corporation) reads on stabilization of radio-labelled compounds. Examples of radio-labelled compounds include e.g. Angiotensin II (5-L-isoleucine) [tyrosyl-<sup>125</sup>I]-(monoiodinated).

Ang II may be turned into potent antagonist or partial antagonists by changes in their amino acid composition. For instance substituting phenylalanine in position 8 with isoleucine and aspartic acid in position 1 with sarcosine changes the peptide into a potent antagonist.

The specificity towards the AT<sub>1</sub> receptor may be increased by cyclisation or bridging of the amino acids in position 4 and 6. Similarly introducing sarcosine in position 1 and glycine in position 8 makes the peptide into a AT<sub>1</sub> selective antagonist (See RC Speth. Sarcosine11, glycine8 angiotensin II is an AT<sub>1</sub> angiotensin II receptor subtype selective antagonist). Regulatory peptides 115 (2003) 203-209)

As mentioned above, the natural ligand to the AT<sub>1</sub> receptor is the octapeptide AngII, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, which binds to the AT<sub>1</sub> receptor in the nano-mole range.

When modifying a naturally binding ligand to a receptor by the binding of a moiety, in particular with moieties that are relatively large and relatively bulky, the affinity of the peptide vector is frequently compromised.

We have surprisingly found that the octapeptide AngII, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe and derivatives thereof, when substituted in specific positions of the peptide not only retain its binding capability but surprisingly increases its affinity for the Angiotensin II receptors, in particular to the AT<sub>1</sub> receptor.

### Summary of the Invention

A first objective with the present invention is to provide pharmaceuticals useful in the treatment of heart failure, cardiac arrhythmias and other diseases where fibrosis is prominent such as in COPD, liver fibrosis, and atherosclerosis comprising a

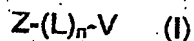
targeting moiety that demonstrate a higher binding affinity to the AT<sub>1</sub> receptor than the native octapeptide Ang II. The pharmaceutical should demonstrate antagonistic activity.

A second objective with the present invention is to provide pharmaceuticals useful in the diagnosis of heart failure and other diseases where fibrosis is prominent such as in COPD, liver fibrosis, and atherosclerosis comprising a targeting moiety incorporating an imageable moiety. The imageable moiety can be any imageable moiety which when administered to a subject can generate an image of at least a part of said subject to which said contrast agent has distributed, e.g. by radio imaging, SPECT, PET, MRI, X-ray, optical imaging (OI), ultrasound (US), electrical impedance or magnetometric imaging modalities. The targeting moiety incorporating the imageable modality should demonstrate a higher binding affinity to the AT<sub>1</sub> receptor than the Ang II and should preferably act as an antagonist although a weak agonistic activity may also be acceptable.

The new pharmaceuticals can hence be used sequentially or concurrently as a therapeutic agent when carrying a suitable imageable moiety for diagnostic imaging.

Further objectives comprise providing methods of treatment of hypertension, fibrosis, COPD and related diseases and methods of imaging of heart failure and fibrosis and also methods of monitoring of progression of treatment for such diseases and disorders as well as for related vascular diseases and disorders. The invention further provides novel pharmaceutical compositions and precursors for the preparation of diagnostic agents. Kits of diagnostic agents, in particular kits for the preparation of radiopharmaceutical diagnostic agents are also provided.

The pharmaceuticals of the invention comprise a peptide V, optionally a linker L and a moiety Z and can be visualised by the formula (I)



wherein

V denotes a peptide with a binding sequence -X<sup>1</sup>-X<sup>2</sup>-Val-Tyr-Ile-His-Pro-X<sup>3</sup>,

L denotes an optional linker,

Z denotes a group that optionally can carry an imaging moiety M,

n is 0 or 1,

X<sup>1</sup> denotes an amino acid,

X<sup>2</sup> denotes Arg or N-alkylated Arg, or a mimetic of Arg ,

X<sup>3</sup> denotes an amino acid containing a hydrophobic side-chain,

and wherein the residues Val and Ile at position 3 and 5 respectively may optionally be replaced with amino acids capable of forming a bridge,

Z forms a bond with the amino acid X<sup>1</sup> optionally through the linker L, and

M where present denotes an imageable moiety capable of detection either directly or indirectly in an diagnostic imaging procedure.

### Detailed Description of the Invention

The invention is described in the patent claims. Specific features of the invention are outlined in the following detailed description and the Examples.

In the targeting moiety of the formula (I) above V denotes the peptide sequence -X<sup>1</sup>-X<sup>2</sup>-Val-Tyr-Ile-His-Pro-X<sup>3</sup>.

In the peptide V of formula (I) the amino acids are L-amino acids as in the native Ang II where not defined otherwise.

The three letter abbreviations used for the amino acids have the following meaning:

Arg	- Arginine
Asp	- Aspartic acid
Cys	- Cysteine
Hcy	- Homocysteine
Gly	- Glycine
Sar	- Sarcosine
Val	- Valine
Tyr	- Tyrosine
Ile	- Isoleucine
His	- Histidine
Pro	- Proline
Phe	- Phenylalanine
Abu	- 2-Amino-butyric acid
Nva	- 2-Amino-pentanoic acid
Nle	- 2-Amino-hexanoic acid
Phg	- 2-Amino-2-phenyl acetic acid

- Hph - 2-Amino-4-phenyl butanoic acid  
 Bip - 2-Amino-3-biphenyl propionic acid  
 Nal - 2-Amino-3-naphthyl propionic acid  
 Cha - 2-Amino-3-cyclohexyl propionic acid

The amino acids of V preferably are independently selected such that  $X^1$  denotes  $-NY_1-(CH_2)_m-CO-$  where  $m$  is an integer from 1 to 10 and  $Y_1$  is H or an alkyl or aryl containing substituent, most preferred Gly  
 $X^2$  denotes Arg or N-Methyl-Arg or the Arg mimetics Phe[4-guanidino] and Gly-4-piperidyl[N-amidino],  
 $X^3$  denotes Phe, D-Phe, Ile, Abu, Nva, Nle, Phg; Hph, Bip, Nal or Cha, most preferred D-Phe, Bip, Ile or Hph.

Still further preferred are pharmaceuticals where  $X^1$  denotes Gly,  $X^2$  denotes Arg and  $X^3$  denotes Ile.

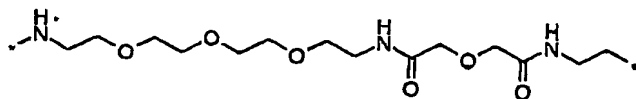
If the amino acids at position 3 and 5 are selected to form a bridging unit the bridge preferably containing a  $-CH_2-CH_2-$ ,  $-S-CH_2-$ ,  $-S-CH_2-S-$ , lactam or  $-S-S-$  unit. More preferred the covalent bond is a disulfide bond formed by oxidation of 2 cysteine or homocysteine pairings.

Examples of suitable linkers L are described in WO 98/18496 and in WO 01/77145 (pages 23 to 27) the content of which are hereby incorporated by reference. L may preferably represent a polyalkylene glycol unit such as polyethylene glycol (PEG) and polypropylene glycol (PPG), a carbohydrate, dextran or 1 to 10 amino acids. The linker may also serve as a biomodifier as described e.g. in WO 03/006491.

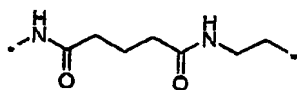
The linker L may also be derived from alkylamines or arylamines preferably compounds of the formula  $NH-(CH_2)_m$  optionally combined with  $-CO-(CH_2)_m-CO-$  where  $m$  denotes a positive integer from 1 to 10.

The linker may also comprise one or more units of PEG as defined in formula IV hereinafter wherein  $n$  is an integer from 1 to 10.

Most preferred are linkers defined by formula (V) and (VI):



Formula (V)



Formula (VI)

The moiety Z comprises a non-peptidic moiety having a molecular weight of more than 50 D (Dalton), more preferably between 100 and 1000 D and still more preferably between 300 to 700 D. The moiety Z can be any pharmaceutically acceptable chemical entity provided that the resulting targeting moiety of formula (I) demonstrates a higher binding affinity to the AT<sub>1</sub> receptor than the native ligand Ang II. More specifically Z denotes an organic group having a suitable functional group such that Z can be reacted either with the linker L or directly with the peptide V to form a stable covalent bond.

Z can denote a straight or branched hydrocarbyl group optionally containing one or more double or triple bonds and optionally substituted by halogen, oxygen, sulphur or phosphorous atoms or optionally including heteroatoms such as oxygen, nitrogen or sulphur. More specifically the hydrocarbyl group can denote a substituted or unsubstituted alkyl, alkenyl, alkynyl groups having a molecular weight of at least 50 D.

Z can also denote one or more linked carbocyclic residues comprising monocyclic, bicyclic or tricyclic ring systems which can be saturated, partially unsaturated or aromatic and which can be substituted or unsubstituted and having a molecular weight of at least 50 D. Examples of such ring systems are aryl, aralkyl, cyclohexyl, adamantyl and naphthyl.

Z can further denote one or more linked heterocyclic compounds such as 5, 6, 7, 8, 9 or 10-membered ring systems which can be monocyclic, bicyclic or tricyclic and can contain one or more N, O, S and P as heteroatoms. Such ring systems can also be linked to hydrocarbyl and carbocyclic groups and defined above or fused to

carbocyclic groups. Examples of such groups acridinyl, benzofuranyl, indolyl, pyridyl, piperidinyl, morphoridinyl and thienyl.

Z may also denote a polyalkylene glycol such as polyethylene glycol (PEG) and polypropylene glycol (PPG), a carbohydrate such as mono or polysaccharides all having a molecular weight in excess of 50 D. Polyalkylene glycols can additionally act as biomodifiers.

Specifically Z denotes a chelating agent such as acyclic or cyclic polyaminocarboxylates (e.g. DTPA, DTPA-BMA, DOTA and DO3A) as described e.g. in US patent 4 647 447 (Schering AG) and WO 86/02841 (Nycomed Salutar, Inc.) which is hereby incorporated by reference. Further chelating agents comprises aminethiols such as diaminedithiols, amineoximes and hydrazines and related agents as described in WO 01/77145 (see Table I therein) which is hereby incorporated by reference. The chelating agent cPN216 is particularly preferred.

For pharmaceuticals useful in treatment Z can be any entity as described above.

For pharmaceuticals useful in diagnosis and particularly in *in vivo* diagnosis the moiety Z must be able to carry the imageable moiety or moieties denoted M. By carrying is meant any form of association between the moiety Z and M such as a chemical bond, e.g. covalent bond or electrovalent or ionic bonds or by absorption or any other type of association.

In a preferred embodiment one moiety Z is covalently bound directly to X<sup>1</sup> forming an N-alkyl glycine unit.

Chelating agents of formula (VII) and (VIII) hereinafter are also particularly preferred.

M can be any imageable moiety. The nature of M will depend of the imaging modality utilised in the diagnosis. M must be capable of detection either directly or indirectly in an *in vivo* diagnostic imaging procedure. eg. moieties which emit or may be caused to emit detectable radiation (eg. by radioactive decay, fluorescence excitation, spin resonance excitation, etc.), moieties which affect local electromagnetic fields (eg. paramagnetic, superparamagnetic, ferrimagnetic or ferromagnetic species), moieties which absorb or scatter radiation energy (eg. chromophores, particles (including gas

or liquid containing vesicles), heavy elements and compounds thereof, etc.), and moieties which generate a detectable substance (eg. gas microbubble generators).

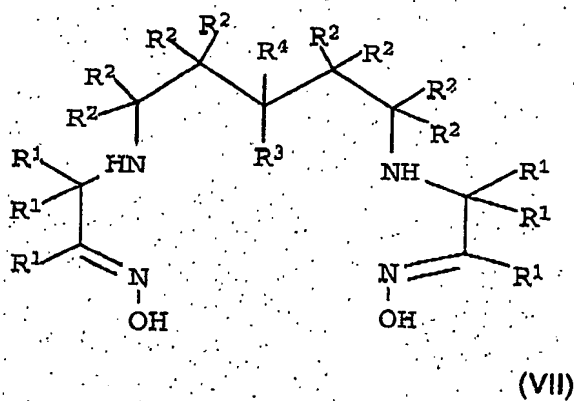
A wide range of suitable imageable moieties are known from e.g. WO 98/18496, the content of which is incorporated by reference.

Imaging modalities and imageable moieties M are described in more detail hereinafter:

In a first embodiment, the compound of formula (I) comprises a moiety Z carrying one or more imageable moieties M useful in the Radio and SPECT imaging modality. Preferably M is a gamma emitter with low or no alpha- and beta-emission and with a half-life of more than one hour. Preferred groups M are the radionuclides  $^{67}\text{Ga}$ ,  $^{111}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{81\text{m}}\text{Kr}$ ,  $^{99}\text{Mo}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{201}\text{Tl}$  and  $^{133}\text{Xe}$ . Most preferred is  $^{99\text{m}}\text{Tc}$ .

When M denotes a metallic radionuclide then Z denotes a chelating agent suitable for forming a stable chelate with M. Such chelating agents are well known from the state of art and typical examples of such chelating agents are described in Table I of WO 01/77145.

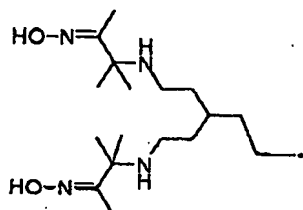
Particularly preferred are chelating agents of formula (VII):



wherein:

each  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and  $\text{R}^4$  is independently H or  $\text{C}_{1-10}$  alkyl,  $\text{C}_{3-10}$  alkylaryl,  $\text{C}_{2-10}$  alkoxyalkyl,  $\text{C}_{1-10}$  hydroxyalkyl,  $\text{C}_{1-10}$  alkylamine,  $\text{C}_{1-10}$  fluoroalkyl, or 2 or more R groups, together with the atoms to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring.

More particularly preferred are chelating agents of formula (VII) where  $R^1$ ,  $R^2$  and  $R^3$  are hydrogen or methyl groups and  $R^4$  is an alkylamine group, most specifically a compound of formula (VIII), herein denoted cPN126.



Formula (VIII)

Most preferred for Z is when the chelate is cPN216 then the imaging moiety M is  $^{99m}\text{Tc}$ .

Non-metal radionuclides such as  $^{123}\text{I}$ ,  $^{125}\text{I}$  and  $^{131}\text{I}$  may be covalently linked to the moiety Z by a substitution or addition reaction well known from the state of art.

In a second embodiment, the compound of formula (I) comprises a moiety Z carrying one or more imageable moieties M useful in the PET imaging modality. M then denotes a radioemitter with positron-emitting properties. Preferred groups M are the radionuclides  $^{11}\text{C}$ ,  $^{18}\text{F}$ ,  $^{68}\text{Ga}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$  and  $^{82}\text{Rb}$ .  $^{18}\text{F}$  is specifically preferred.

When M denotes a metallic radionuclide then Z denotes a chelating agent suitable for forming a stable chelate with M. Such chelating agents are well known from the state of art and typical examples of such chelating agents are described in Table I of WO 01/77145 and to the previous part on Radio and SPECT imaging.

In another preferred embodiment Z is the DOTA chelating agent and M is  $^{68}\text{Ga}$  which can be readily introduced in to the chelate using microwave chemistry.

Non-metal radionuclides such as  $^{18}\text{F}$  may be covalently linked to the moiety Z by a substitution or addition reaction well known from the state of art and also described eg. in WO03/080544 which is hereby incorporated by reference.

In a third embodiment, the compound of formula (I) comprises a moiety Z carrying one or more imageable moieties M useful in the MR imaging modality. M here

denotes a paramagnetic metal such those mentioned in US patent 4 647 447,  $Gd^{3+}$ ,  $Dy^{3+}$ ,  $Fe^{3+}$  and  $Mn^{2+}$  are particularly preferred and Z denotes a chelating agent, in particular a chelating agent such as acyclic or cyclic polyaminocarboxylates (e.g. DTPA, DTPA-BMA, DOTA and DO3A) as described e.g. in US patent 4 647 447 and WO 86/02841. M may also denote metal oxides such as superparamagnetic, ferrimagnetic or ferromagnetic species which are absorbed by Z, e.g. such that Z function as a coating to the metal oxide. Metal oxides for use as MR contrast agents are described e.g. in US patent 6 230 777 which is hereby incorporated by reference.

In a fourth embodiment the compound of formula (I) comprises a moiety Z carrying one or more imageable moieties M useful in the X-ray imaging modality. M here denotes a heavy metal such as W, Au and Bi preferably in the form of oxides which may be absorbed to Z. Iodinated aryl derivatives are particularly well known as X-ray contrast agents, e.g. Iopamiron™ and Omnipaque™. These agents can be linked via their amide or amine functions to the peptide V.

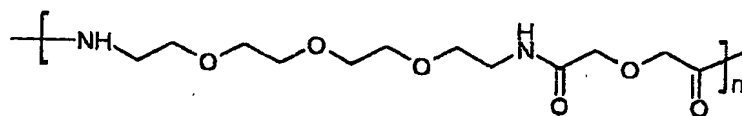
Ultrasound imaging agents in the form of gas filled microvesicles can be utilised in the imaging of receptors e.g. when they are functionalised for binding to a peptide as described in the state of art e.g. in WO98/18500.

Use of contrast agents for optical imaging with affinity for biological targets can be provided following the procedures in the state of art e.g. as described in WO 96/17628 of Schering AG which is herewith incorporated by reference.

The pharmaceutical of formula (I) may be further modified by the attachment of one or more biomodifier groups such as polyalkylene glycol unit e.g. polyethylene glycol (PEG) and polypropylene glycol (PPG). Examples of biomodifier groups are described in WO 03/006491 the content of which is hereby incorporated by reference. The biomodifier groups can be linked to any position in the compound of formula (I) as long as it does not significantly affect in a negative way the compound's ability to link to the target receptor.

The biomodifier is preferably based on a monodisperse PEG building block comprising 1 to 10 units of said building block, said biomodifier having the function of modifying the pharmacokinetics and blood clearance rates of the said agents. In a preferred embodiment of this invention, the compound of formula IV, represents a

biomodifier unit comprised of polymerisation of the monodisperse PEG-like structure, 17-amino-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid.

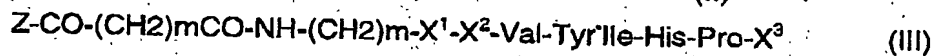
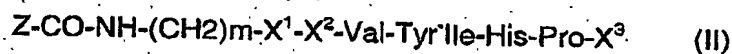


(IV)

wherein  $n$  equals an integer from 1 to 10 and where the C-terminal unit forms an amide bond.

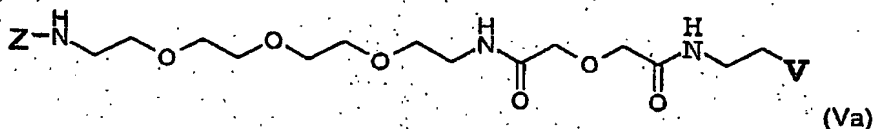
Examples of pharmaceuticals of formula (I) are represented by:

Compounds of the formula (II) and (III):

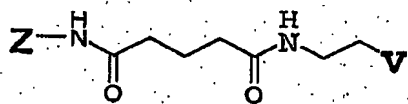


where  $m$  is an integer of between 1 and 5 and  $Z$ ,  $X^1$ ,  $X^2$  and  $X^3$  are as defined previously.

Compounds defined by formula Va and VIa:



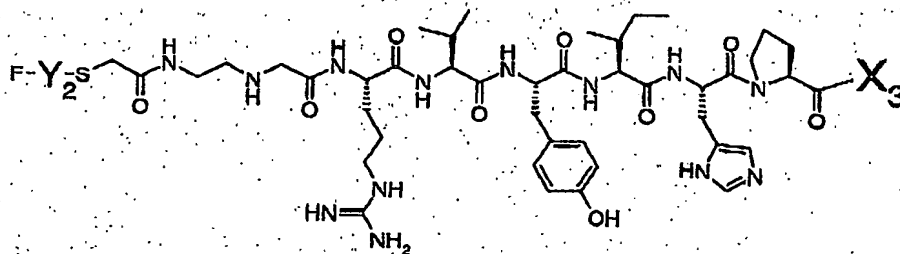
(Va)



(VIa)

where  $Z$  and  $V$  are as defined previously.

Compounds of the formula (IX):



(IX)

where  $Y^2$  is alkyl, aryl or a short PEG containing moiety and  $Y^2$  is preferably  $-CH_2-$   
 $CH_2-CH_2-$  and  $X^3$  is as defined above.

Preferred examples of pharmaceuticals of formula (I) are represented by:

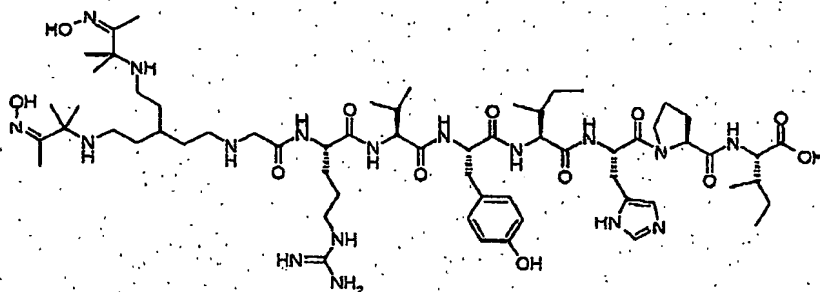
cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH

cPN216-Gly-MeArg-Val-Tyr-Ile-His-Pro-D-Phe-OH

cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Bip-OH

As noted above, for use in in vivo diagnosis the chelates of these compounds with  $^{99m}Tc$  or  $^{18}F$  are particularly preferred.

Specifically preferred is the compound cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH the chemical structure of which is visualised in formula (X):



Formula (X)

and its chelate with  $^{99m}Tc$ .

The pharmaceuticals of formula (I) are preferably administered as a pharmaceutical formulation comprising the compound of formula (I) in a form suitable for administration to a mammal, such as a human. The administration is suitable carried out by injection or infusion of the formulation such as an aqueous solution. The formulation may contain one or more pharmaceutical acceptable additives and/or excipients e.g. buffers; solubilisers such as cyclodextrins; or surfactants such as Pluronic, Tween or phospholipids. Further, stabilisers or antioxidants such as ascorbic acid, gentisic acid or para-aminobenzoic acid and also bulking agents for lyophilisation such as sodium chloride or mannitol may be added.

In a still further aspect a kit for the preparation of a radiopharmaceutical composition of formula (I) comprising a peptide-chelate conjugate and a reducing agent is provided. Preferably the reducing agent of the kit is a stannous salt. The kit may also comprise one or more stabilisers, antioxidants, bulking agents for lyophilisation and solubilisers.

General procedures for the preparation of the pharmaceuticals and its precursors

The abbreviations used have the following meanings:

Fmoc: 9-fluorenylmethoxycarbonyl

Boc: t-butyloxycarbonyl

tBu: t-butyl

Trt: trityl

Pmc: 2,2,5,7,8-pentamethylchroman-6-sulfonyl

TFA: trifluoroacetic acid

HBTU: O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate

DMF: dimethylformamide

NMP: N-methylpyrrolidone

TIS: triisopropylsilane

NHS: N-hydroxysuccinimide

NMM: N-methylmorpholine

RP-HPLC: reversed phase high pressure liquid chromatography

Wang resin: p-benzyloxybenzyl alcohol resin

**Synthesis of V:**

The peptides V of the present invention can be synthesised using all the known methods of chemical synthesis but particularly useful is the solid-phase methodology of Merrifield employing an automated peptide synthesizer (J. Am. Chem. Soc., 85: 2149 (1964)). Typically, the desired sequences are assembled by solid-phase peptide synthesis. Standard procedures for the synthesis strategy employed for the examples of this invention are described in E. Atherton & R.C. Sheppard, "Solid phase peptide synthesis: a practical approach", 1989, IRL Press, Oxford.

For example, a resin with an acid-labile linker group, to which the desired amino-protected C-terminal amino acid residue has been esterified, is used. The amino protecting group is then removed and the second amino acid in the sequence is coupled using a suitable condensation reagent. Amino acids with semi-permanent amino protecting groups and permanent protecting groups for the functional side

chains are employed. Amino-deprotection and coupling cycles are then repeated in alternating steps until the sequence of interest is assembled.

Alternatively, the peptides V can be synthesised through solution peptide synthesis methods known in the art, either in a step-wise manner from the carboxyl terminus and/or through the application of segment condensation or ligation methods, employing comprehensive or minimal protection strategies. Combined solution-solid phase segment condensation approaches can also be applied.

Generally, the reactive side-chain groups present (for example amino, hydroxyl, guanidino and carboxyl groups) will be protected during overall synthesis as indicated above. A wide choice of protecting groups for amino acids is known (see, e.g., Greene, T.W. & Wuts, P.G.M. (1991) *Protective groups in organic synthesis*, John Wiley & Sons, New York). Amino protecting groups which may be employed include 9-fluorenylmethoxycarbonyl (Fmoc) and *t*-butoxycarbonyl (Boc). Side-chain protecting groups which may be employed include *t*-butyl (tBu), trityl (Trt), Boc, and 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc). It will be appreciated that a wide range of other such groups are known in the art.

Finally the permanent side-chain protecting groups are removed and the peptide is cleaved from the resin, usually simultaneously through treatment with a suitable acidic reagent, e.g. trifluoroacetic acid (TFA).

#### Conjugation of L to V:

L can be conjugated to V using all the known methods of chemical synthesis. Particularly useful is the nucleophile substitution reaction where a leaving group on the peptide N-terminus is replaced by a nucleophilic group on L. Such a leaving group may be a bromide attached in alpha position to a carbonyl group, and such a nucleophile may be nitrogen.

#### Conjugation of Z to V or to L:

Z can be conjugated directly to V using the same methods as for the conjugation of L to V. In the case where Z is attached to V via L any methods of chemical synthesis may be used in the conjugation of Z and L. Particularly useful is amide bond formation.

# SEQUENCE LISTING

<110> Amersham Health AS

<120> Angiotensin II analogues

<130> PN0380

<160> 5

<170> PatentIn version 3.1

<210> 1

<211> 8

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesised peptide

<220>

<221> misc\_feature

<222> (1)..(1)

<223> Any amino acid

<220>

<221> misc\_feature

<222> (2)..(2)

<223> Arg, N-alkylated Arg or a mimetic of Arg

<220>

<221> misc\_feature

<222> (8)..(8)

<223> Amino acid containing a hydrophobic side chain

<400> 1

Xaa Xaa Val Tyr Ile His Pro Xaa

1 5

<210> 2

<211> 8

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesised angiotensin II

<400> 2

Asp Arg Val Tyr Ile His Pro Phe

1 5

<210> 3

<211> 8

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesised peptide

<400> 3

Gly Arg Val Tyr Ile His Pro Ile

1 5

<210> 4

<211> 8

<212> PRT

<213> Artificial sequence

<220>

<223> Synthesised peptide

<220>

<221> MISC\_FEATURE

<222> (2)..(2)

<223> Methylated Arg

<220>

<221> MISC\_FEATURE

<222> (8)..(8)

<223> D-Phe

<400> 4

Gly Xaa Val Tyr Ile His Pro Xaa

1 5

<210> 5

<211> 8

<212> PRT

<213> Artificial sequence.

<220>

<223> Synthesised peptide

<220>

<221> MISC\_FEATURE

<222> (8)..(8)

<223> Bip-2-amino-3-biphenyl propionic acid

<400> 5

Gly Arg Val Tyr Ile His Pro Xaa

1 5

## Examples:

### Example 1 Ang II analogues with dye for optical imaging

#### Fluorescein-NH-(CH<sub>2</sub>)<sub>2</sub>-Gly-Arg-Val-Ile-His-Pro-Ile-OH

A peptide analogue of Ang II was synthesised on an Applied Biosystems 433A peptide synthesizer starting with 0.1 mmol Fmoc-Ile-Wang resin. An excess of 1 mmol pre-activated amino acids (using O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU)) was applied in the coupling steps up to Arg. The N-terminus was bromoacetylated using 0.5 mmol bromoacetic anhydride in DMF for 30 minutes. The bromoacetylated resin was then treated with a solution of 0.5 mmol N-Boc-ethylenediamine dissolved in N-methylpyrrolidone (NMP) for 30 minutes. The simultaneous removal of side-chain protecting groups and cleavage of the peptide from the resins was carried out in 5 mL TFA containing 2.5 % triisopropylsilane (TIS) and 2.5 % water for two hours. TFA is removed in vacuo, diethyl ether added to the residue and the precipitated peptide washed with diethyl ether and air-dried.

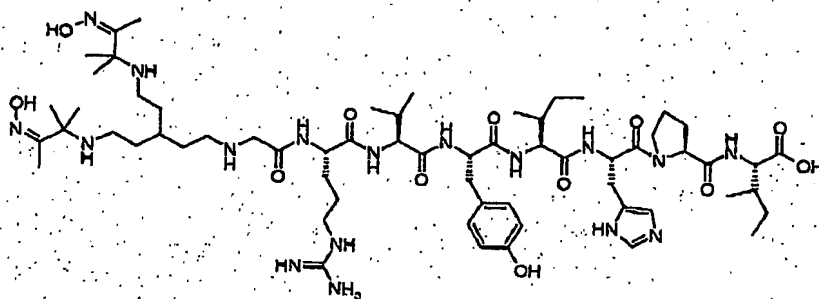
23 mg of crude peptide, 16 mg of fluorescein NHS ester and 12  $\mu$ L of N-methylmorpholine (NMM) were dissolved in dimethylformamide (DMF) and the reaction mixture stirred over night. Purification of the reaction product by preparative RP-HPLC (Conditions: 5-50 % B over 40 min, where A = H<sub>2</sub>O/0.1 % TFA and B = CH<sub>3</sub>CN/0.1 % TFA; flow rate, 10 mL/min; column, Phenomenex Luna 5 $\mu$  C18 (2) 250 x 21.20 mm) afforded 19 mg of pure fluorescein-peptide conjugate. The peptide was analysed by analytical HPLC (Conditions: Gradient, 5-50 % B over 10 min where A = H<sub>2</sub>O/0.1 % TFA and B = CH<sub>3</sub>CN/0.1 % TFA; flow, 1 mL/min; column, Phenomenex Luna 3 $\mu$  C18 (2) 50 x 4.6 mm; detection, UV 214 nm; product retention time 8.91 min). Further product characterisation was carried out using electrospray mass spectrometry (MH<sup>+</sup> calculated, 1355.6; MH<sup>+</sup> found, 1355.2).

In a similar manner Cy5.5 NHS ester (a dye supplied from Amersham Biosciences) may be used to synthesis the dye-peptide conjugate Cy5.5-NH-(CH<sub>2</sub>)<sub>2</sub>-Gly-Arg-Val-Ile-His-Pro-Ile-OH.

$$^{18}\text{F}-(\text{CH}_2)_3\text{-S-CH}_2\text{CO-NH-(CH}_2)_2\text{-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH}$$

The crude peptide is treated with one equivalent of chloroacetic anhydride dissolved in DMF for 30 min. The chloroacetylated peptide is purified using preparative RP-HPLC.  $^{18}\text{F}$ -propyl thiol is conjugated to the pure chloroacetylated peptide to afford a  $^{18}\text{F}$ -labelled peptide for PET imaging.

cPn216-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH



A peptide analogue of Ang II was synthesised on an Applied Biosystems 433A peptide synthesizer starting with 0.1 mmol Fmoc-Ile-Wang resin. An excess of 1 mmol pre-activated amino acids (using HBTU) was applied in the coupling steps up to Arginine. The N-terminus was bromoacetylated using 0.5 mmol bromoacetic anhydride in DMF for 30 minutes. The bromoacetylated resin was then treated with a solution of 0.2 mmol cPn216 and 0.4 NMM dissolved in DMF for 16 hours.

The simultaneous removal of side-chain protecting groups and cleavage of the peptide from the resins was carried out in 5 mL TFA containing 5 % TIS, 5 % water and 2.5 % phenol for two hour. TFA is removed in vacuo, diethyl ether added to the residue and the precipitated product washed with diethyl ether and air-dried.

Purification by preparative RP-HPLC (0-30 % B over 40 min, where A = H<sub>2</sub>O/0.1 % TFA and B = CH<sub>3</sub>CN/0.1 % TFA, at a flow rate of 10 mL/min on a Phenomenex Luna 5 $\mu$  C18 (2) 250 x 21.20 mm column) of the product afforded 12 mg pure chelate-peptide conjugate. The product was analysed by analytical HPLC (Conditions: Gradient, 5-50 % B over 10 min where A = H<sub>2</sub>O/0.1 % TFA and B = CH<sub>3</sub>CN/0.1 % TFA; flow, 2 mL/min; column, Phenomenex Luna 3 $\mu$  C18 (2) 50 x 4.6 mm; detection, UV 214 nm; product retention time 7.02 min). Further product characterisation was carried out using electrospray mass spectrometry (MH<sup>+</sup> calculated, 1280.8; MH<sup>+</sup> found, 1280.5).

#### Example 4    Ang II analogue for <sup>99m</sup>Tc labelling

cPn216-CO-(CH<sub>2</sub>)<sub>3</sub>CO-NH-(CH<sub>2</sub>)<sub>2</sub>-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH

A peptide analogue of Ang II was synthesised on an Applied Biosystems 433A peptide synthesizer starting with 0.15 mmol Fmoc-Ile-Wang resin. An excess of 1 mmol pre-activated amino acids (using HBTU) was applied in the coupling steps up to Arginine. The N-terminus was bromoacetylated using 0.75 mmol bromoacetic anhydride in DMF for 30 minutes. The bromoacetylated resin is then treated with a solution of 0.75 mmol N-Boc-ethylenediamine dissolved in NMP for 60 minutes.

The simultaneous removal of side-chain protecting groups and cleavage of the peptide from the resins was carried out in 10 mL TFA containing 2.5 % TIS and 2.5 % water for 90 minutes. TFA is removed in vacuo, diethyl ether added to the residue and the precipitated product washed with diethyl ether and air-dried.

5.5 mg of peptide, 23 mg of cPn216 tetrafluorothiophenyl ester and 10  $\mu$ L of NMM were dissolved in DMF and the reaction mixture stirred for 3 hrs. Purification by preparative RP-HPLC (conditions: 0-30 % B over 40 min, where A = H<sub>2</sub>O/0.1 % TFA and B = CH<sub>3</sub>CN/0.1 % TFA; flow rate, 10 mL/min; column, Phenomenex Luna 5 $\mu$  C18 (2) 250 x 21.20 mm) of the product afforded 4.3 mg pure chelate-linker-peptide conjugate. The product was analysed by analytical HPLC (Conditions: Gradient, 5-50 % B over 10 min where A = H<sub>2</sub>O/0.1 % TFA and B = CH<sub>3</sub>CN/0.1 % TFA; flow, 0.3 mL/min; column, Phenomenex Luna 3 $\mu$  C18 (2) 50 x 2 mm; detection, UV 214 nm;

product retention time 6.51 min). Further product characterisation was carried out using electrospray mass spectrometry ( $MH^+$  calculated, 1436.9;  $MH^+$  found, 1436.7).

Example 5 Antiarrhythmic effect in a post Myocardial Infarction (MI) pig model

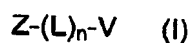
The pharmaceutical cPn216-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH (X) was examined according to the following procedure:

LAD was occluded above the 2<sup>nd</sup> branch and one of the collaterals was occluded as well. Arrhythmia developed as in the first pig and the stabilisation of the condition occurred after injection of 0.5 mg of the compound of formula (X). An initial rise in blood pressure was noticed. Ventricular extrasystoles reappeared after about 15 minutes and a new injection of 0.5 mg of the compound of formula (X) was given. Again the condition stabilised with regular sinus rhythm. During the following hour mainly stable sinus rhythm and a slightly reduced but constant blood pressure were recorded. The pig was then given a lethal dose of potassium chloride to terminate the experiment.



Claims:

1. Pharmaceuticals *characterised by* the formula (I)



wherein

V denotes a peptide with a binding sequence  $-X^1-X^2\text{-Val-Tyr-Ile-His-Pro-}X^3$ ,

L denotes an optional linker,

Z denotes a group that optionally can carry an imaging moiety M,

n is 0 or 1,

$X^1$  denotes an amino acid,

$X^2$  denotes Arg or N-alkylated Arg or a mimetic of Arg ,

$X^3$  denotes an amino acid containing a hydrophobic side-chain,

and wherein the residues Val and Ile at position 3 and 5 respectively may optionally be replaced with amino acids capable of forming a bridge,

Z forms a bond with the amino acid  $X^1$  optionally through the linker L, and

M where present denotes an imageable moiety capable of detection either directly or indirectly in a diagnostic imaging procedure.

2. Pharmaceuticals of claim 1 characterised by having affinity to the Angiotensin II Receptor, particularly to the  $AT_1$  receptor

3. Pharmaceuticals of claims 1 and 2 having antagonistic activity

4. Pharmaceuticals of claims 1 to 3 for the use in therapy wherein M is not present

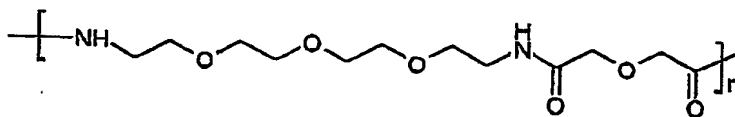
5. Pharmaceuticals of the preceding claims useful in the treatment of heart failure, cardiac arrhythmias and other diseases where fibrosis is prominent.

6. Pharmaceuticals of claim 5 useful in the treatment of COPD, liver fibrosis and atherosclerosis.

7. Pharmaceuticals of claims 1 to 3 for the use in diagnosis wherein M is an in vivo imageable moiety

8. Pharmaceuticals of claims 1 to 3 and 7 useful in the diagnosis of heart failure and other diseases where fibrosis is prominent.
9. Pharmaceuticals of the preceding claims wherein the amino acids are L-amino acids where not defined otherwise
10. Pharmaceuticals of the preceding claims wherein the amino acids of  $X^1$  to  $X^3$  are independently selected from  
 $X^1$  denoting  $-NY_1-(CH_2)_m-CO-$  where  $m$  is an integer from 1 to 10 and  $Y_1$  is H or an alkyl or aryl containing substituent,  
 $X^2$  denoting Arg or N-Methyl-Arg or the Arg mimetics Phe[4-guanidino] and Gly-4-piperidyl[N-amidino],  
 $X^3$  denoting Phe, D-Phe, Ile, Abu, Nva, Nle, Phg, Hph, Bip, Nal or Cha,
11. Pharmaceuticals of claim 10 wherein the amino acids of  $X^1$  to  $X^3$  are independently selected from  
 $X^1$  denoting Gly  
 $X^2$  denoting Arg or N-Methyl-Arg  
 $X^3$  denoting D-Phe, Bip, Ile or Hph
12. Pharmaceuticals of claim 11 wherein the amino acids are L-amino acids and where  $X^1$  is Gly,  $X^2$  is Arg and  $X^3$  is Ile.
13. Pharmaceuticals of the preceding claims wherein the amino acids at position 3 and 5 are selected to form a bridging unit the bridge preferably containing a  $-CH_2-CH_2-$ ,  $-S-CH_2-$ ,  $-S-CH_2-S-$ , lactam or  $-S-S-$  unit.
14. Pharmaceuticals of claim 13 wherein the bridging unit is a disulfide bond formed by oxidation of two Cys or homocysteine pairings.
15. Pharmaceuticals of the preceding claims wherein one or more biomodifier groups are attached to any positions of the compound of formula (I)
16. Pharmaceuticals of claim 15 where the biomodifier groups are selected from polyalkylene glycol units, specifically from polyethylene glycol (PEG) and polypropylene glycol (PPG) units.

17. Pharmaceuticals of claim 15 and 16 where the biomodifier groups are selected from compounds of the formula (IV)



(IV)

wherein n equals an integer from 1 to 10 and where the C-terminal unit is an amide moiety.

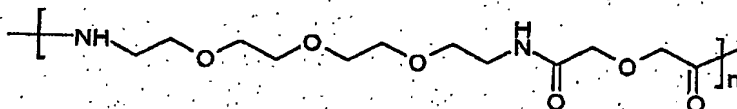
18. Pharmaceuticals of the preceding claims wherein n denotes zero

19. Pharmaceuticals of claims 1 to 17 wherein n denotes 1 and L denotes a polyalkylene glycol unit such as polyethylene glycol (PEG) and polypropylene glycol (PPG), a carbohydrate, dextran or 1 to 5 amino acids

20. Pharmaceuticals of claims 1 to 17 wherein n denotes 1 and L is derived from alkylamines or arylamines.

21. Pharmaceuticals of claim 20 wherein n denotes 1 and L denotes compounds of the formula  $\text{NH}-(\text{CH}_2)_m$ - optionally combined with  $-\text{CO}-(\text{CH}_2)_m-\text{CO}-$  where m denotes a positive integer from 1 to 10.

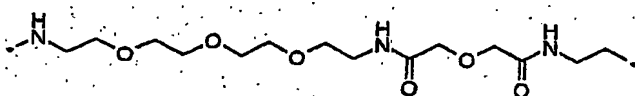
22. Pharmaceuticals of claims 1 to 17 wherein n denotes 1 and L comprises one or more units of PEG of formula (IV)



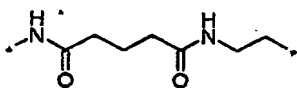
(IV)

wherein n is an integer from 1 to 10.

23. Pharmaceuticals of claims 1 to 17 wherein n denotes 1 and L denotes compounds of formula (V) and (VI):

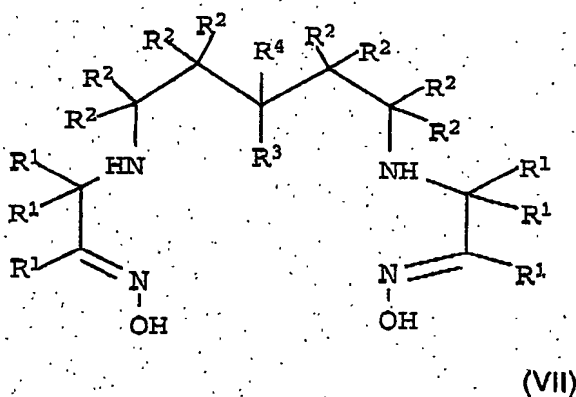


Formula (V)



Formula (VI)

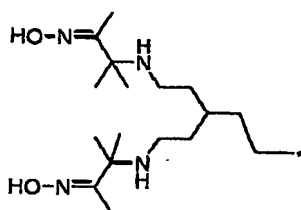
24. Pharmaceuticals of the preceding claims wherein Z has a molecular weight of at least 50 D (Dalton).
25. Pharmaceuticals of claim 24 wherein Z has a molecular weight of 100 D to 1000 D.
26. Pharmaceuticals of claim 25 wherein Z has a molecular weight of 300 D to 700 D.
27. Pharmaceuticals of the preceding claims wherein Z denotes a straight or branched hydrocarbonyl group, a carboxylic residue, a heterocyclic compound, a polyalkylene group or a carbohydrate.
28. Pharmaceuticals of the preceding claims 1 to 26 wherein Z denotes a chelating agent
29. Pharmaceuticals of claim 28 wherein Z denotes a chelating agent of formula (VII)



wherein:

each  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  is independently H or  $C_{1-10}$  alkyl,  $C_{3-10}$  alkylaryl,  $C_{2-10}$  alkoxyalkyl,  $C_{1-10}$  hydroxyalkyl,  $C_{1-10}$  alkylamine,  $C_{1-10}$  fluoroalkyl, or 2 or more R groups, together with the atoms to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring.

30. Pharmaceuticals of claim 29 wherein Z denotes a chelating agent of formula (III)



Formula (VIII)

31. Pharmaceuticals of the preceding claims wherein M represents an imageable moiety for use in diagnosis particularly in *in vivo* diagnosis comprising a moiety which emit or cause to emit detectable radiation, a moiety which affect local electromagnetic fields, moieties which absorb or scatter radiation energy, heavy metals and compounds thereof and moieties which generate a detectable substance.

32. Pharmaceuticals of claim 31 wherein M represents a gamma emitting moiety for Radio or SPECT imaging comprising  $^{67}\text{Ga}$ ,  $^{111}\text{In}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{81\text{m}}\text{Kr}$ ,  $^{99}\text{Mo}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{201}\text{Tl}$  and  $^{133}\text{Xe}$ .

33. Pharmaceuticals of claim 32 wherein M represents  $^{99\text{m}}\text{Tc}$ .

34. Pharmaceuticals of claim 31 wherein M represents a radio emitter with positron emitting properties for PET imaging comprising  $^{11}\text{C}$ ,  $^{18}\text{F}$ ,  $^{68}\text{Ga}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$  and  $^{82}\text{Rb}$ .

35. Pharmaceuticals of claim 34 wherein M represents  $^{18}\text{F}$  or  $^{68}\text{Ga}$ .

36. Pharmaceuticals of claim 31 wherein M represents a paramagnetic metal comprising  $\text{Gd}^{3+}$ ,  $\text{Dy}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Mn}^{2+}$  or a metal oxide such as a superparamagnetic, ferromagnetic or ferromagnetic species.

37. Pharmaceuticals of claim 31 wherein M represents a paramagnetic metal comprising  $\text{Gd}^{3+}$ ,  $\text{Dy}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Mn}^{2+}$  and Z denotes a chelating agent of the group consisting of acyclic or cyclic polyaminocarboxylates, specifically DTPA, DTPA-BMA, DOTA and DO3A.

38. Pharmaceuticals of the preceding claims where M is absent and of the formulas

cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH

cPN216-Gly-MeArg-Val-Tyr-Ile-His-Pro-D-Phe-OH

cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Bip-OH

39. Pharmaceuticals of the preceding claims where M is present and of the formulas

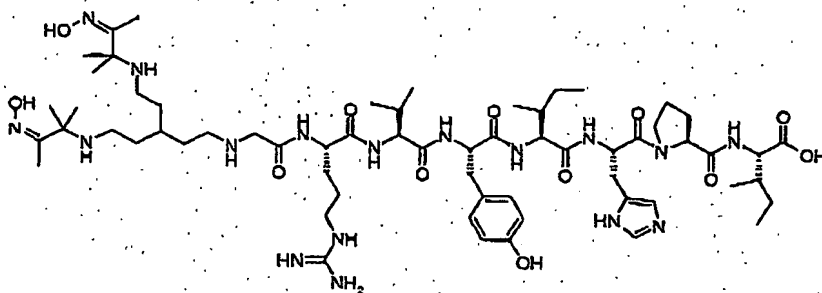
cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Ile-OH

cPN216-Gly-MeArg-Val-Tyr-Ile-His-Pro-D-Phe-OH

cPN216-Gly-Arg-Val-Tyr-Ile-His-Pro-Bip-OH

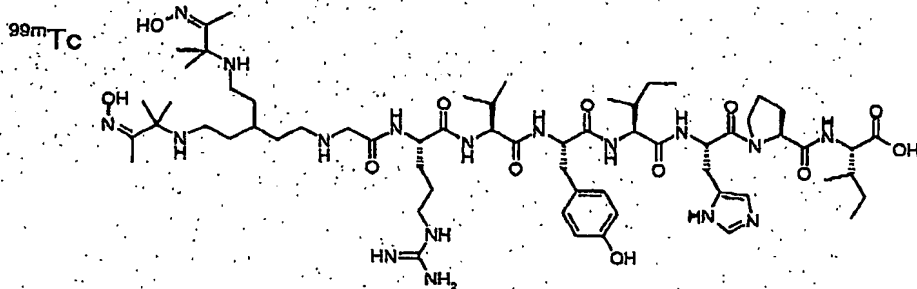
40. Pharmaceuticals of claim 39 where M denotes  $^{99m}\text{Tc}$ .

41. Pharmaceuticals of claim 38 for use in therapy having the formula (X)



Formula (X)

42. Pharmaceuticals of claim 39 and 40 for use as diagnostic agent having the formula (Xa)



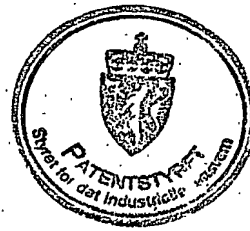
Formula (X)

43. Pharmaceutical precursors for the preparation of diagnostic agents of the formula (I) where M is not present
44. Preparation of diagnostic agents of claim 1 where an imageable moiety M reacted with a pharmaceutical precursor of claim 43.
45. Pharmaceuticals of claim 1 useful for the monitoring of therapeutic treatment
46. Pharmaceutical formulation comprising a compound of formula (I) of claim 1 together with one or more pharmaceutical acceptable additives and/or excipients.
47. Use of pharmaceuticals of claim 1 for the treatment of heart failure, cardiac arrhythmias and other diseases where fibrosis is prominent specifically COPD, liver fibrosis and atherosclerosis.
48. Use of pharmaceuticals of claim 1 for the diagnosis of heart failure and other diseases where fibrosis is prominent specifically COPD, liver fibrosis and atherosclerosis.
49. Method of treatment and diagnosis of heart failure and other diseases where fibrosis is prominent specifically COPD, liver fibrosis and atherosclerosis by the administration of the pharmaceuticals of formula (I) in claim 1
50. Method of claim 49 where the administration of the pharmaceutical is by intravenous injection or infusion into the blood stream
51. Method of in vivo diagnosis of heart failure and other diseases where fibrosis is prominent specifically COPD, liver fibrosis and atherosclerosis in a subject comprising administration of the pharmaceuticals of formula (I) in claim 1 followed by generation of an image of part or all of said subject
52. Method for the monitoring progression of the treatment of heart failure and other diseases where fibrosis is prominent specifically COPD, liver fibrosis and atherosclerosis in a subject comprising administration of pharmaceuticals of formula (I) of claim 1 followed by the generation of an image of part or all of said subject.

53. A kit for the preparation of a radiopharmaceutical composition of formula (I) comprising a peptide-chelate conjugate and a reducing agent.

54. Kit of claim 53 where the reducing agent is a stannous salt.

55. Kit of claim 53 and 54 additionally comprising one or more stabilisers, antioxidants, bulking agents for lyophilisation and solubilisers.



Abstract

The invention comprises pharmaceuticals of formula (I)

$Z-(L)_n-V$  (I)

wherein V denotes a peptide, L denotes an optional linker, Z denotes a group that optionally can carry an imaging moiety M, n denotes 0 or 1. The pharmaceuticals are active as therapeutic agents for the treatment of heart failure, cardiac arrhythmias and diseases where fibrosis is prominent such as COPD, liver fibrosis and atherosclerosis and are also useful as diagnostic agents for the diagnosis of heart failure and diseases where fibrosis is prominent such as COPD, liver fibrosis and atherosclerosis.



Figure 1 - The RAAS system

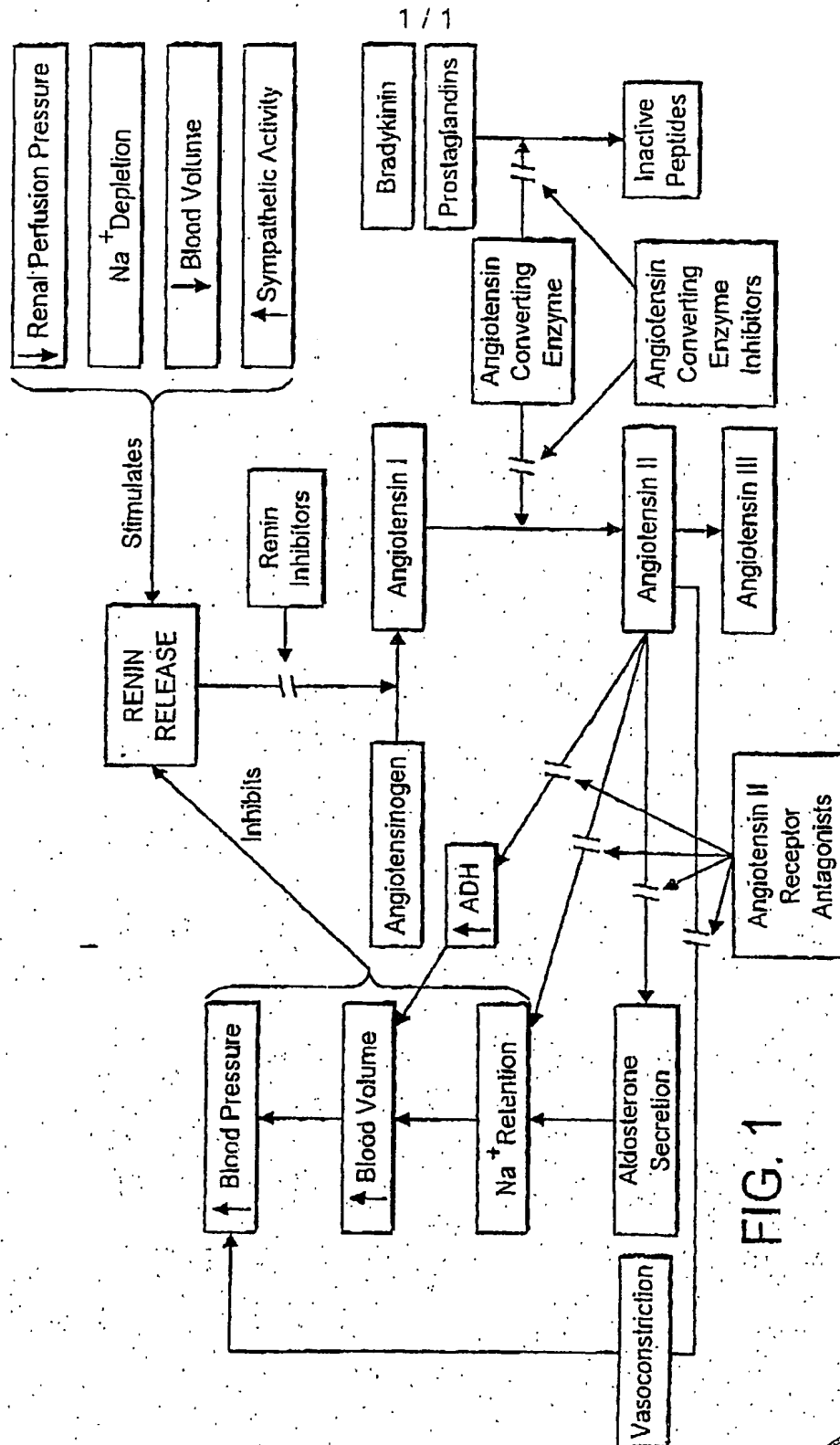


FIG. 1



# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/NO04/000335

International filing date: 05 November 2004 (05.11.2004)

Document type: Certified copy of priority document

Document details: Country/Office: NO  
Number: 20034952  
Filing date: 06 November 2003 (06.11.2003)

Date of receipt at the International Bureau: 25 January 2005 (25.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER: \_\_\_\_\_**

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**